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Poster session A

Eden Parish Hall: Developmental and Cellular Biology/Regeneration

Listed alphabetically by presenting author

The regulatory role of the phosphorylated G protein: Implication of lipid microdomains

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Heterotrimeric G-proteins play crucial roles in various signal transduction pathways, where they act as molecular switches in transducing a signal from G protein-coupled receptors (GPCRs) to downstream effectors. Post-translation modifications such as phosphorylation and palmitoylation can be important factors in regulating G protein function at the plasma membrane, but the mechanism of their involvement remains poorly defined. We used Dictyostelium discoideum as a cellular model that relies on chemotaxis toward a secreted chemoattractant, cyclic adenosine monophosphate (cAMP) during the development phase of their life cycle to examine the roles of G protein palmitoylation and phosphorylation. The Gα2 subunit of D. discoideum is required for the chemotactic response. Our preliminary data demonstrate that the Gα2 subunit is enriched in a lipid raft fraction (LRs). This localization was plamitoyaltion-dependent. We further show that activation significantly shifts Gα2 out of the LRs in an F-actin dependent manner. Once activation occurs, Gα2 is known to be phosphorylated on serine 113. Our goal was to determine if Gα2 phosphorylation has a role in signaling, and its shift from the membrane microdomain. Exchange of serine residue 113 to alanine allows starved cells to quickly begin the aggregation phase compared to wild type, while exchange to aspartic acid showed a dramatic decrease in plasma membrane surface area. In addition, Dictyostelium discoideum 14-3-3 protein has been found by coimmunoprecipitation with activated Gα2. Immunostaining shows some colocalization of the two proteins. This study examines the regulatory function of an activated and phosphorylated Gα2 on its localization to LRs.

Culture of Human Mesenchymal Stem Cells in Norbornene-functionalized Carboxymethylcellulose Hydrogels

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The extracellular environment has been shown to play an important role in regulating cell behavior. Many cell culture systems involve growing cells two-dimensionally in a culture dish, a spatial environment typically not seen by cells in the body. Because of limitations of two-dimensional culture and the importance of the extracellular environment, researchers have been increasingly using three-dimensional matrices for cell culture. The composition of commercially available extracellular matrix solutions are often unknown, presenting problems for investigators. Additionally, commercial matrices offer little ability for spatiotemporal modification, limiting their ability to closely mimic natural processes. We investigated the biocompatibility of a norbornene-functionalized carboxymethylcellulose (NorCMC) hydrogel using human mesenchymal stem cells. These cells maintained high viability in the three-dimensional crosslinked polymer for at least seven days. Studies incorporating functionalized adhesion peptides and cell-degradable crosslinkers are currently being performed.

miR-21 directs the immune response in macrophages during zebrafish cardiac regeneration

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Recent work has demonstrated that macrophages are essential to coordinating tissue regeneration and repair, switching from an early, pro-inflammatory phenotype, towards an anti-inflammatory phenotype later in the regenerative process. Despite the importance these immune cells to regeneration, the mechanisms governing their behavior are incompletely understood. MicroRNAs (miRNAs) are powerful post-transcriptional regulators of gene expression, critical to directing macrophage phenotype. Our lab has identified miR-21 as one of the most highly upregulated miRNAs in response to cardiac injury in the zebrafish. Furthermore, we have demonstrated that miR-21 expression co-localizes spatially and temporally with macrophages during cardiac regeneration. Both experimentally induced miR-21 depletion, as well as macrophage ablation, inhibit cardiomyocyte proliferation during regeneration. RNA-seq studies of the injured zebrafish heart identify immune process genes among the most highly dysregulated in response to LNA antimiR-mediated miR-21 suppression. Together, this work underscores the requirement for upregulation of miR-21 in macrophages during the regenerative process.

Misexpression of CHMP2B in the wing development of Drosophila melanogaster

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The human CHMP2B protein, a member of the ESCRT-III complex, functions in the recycling and degradation of cell surface receptors. It is expressed in neurons in all major regions of the brain. Mutations in the CHMP2B gene can disrupt cell-signaling pathways and can lead to neurodegenerative diseases. An earlier study illustrated photoreceptor neurodegeneration due to misexpression of the CHMP2B protein in the eye of Drosophila melanogaster, suggesting the role of this protein is highly conserved, affecting a variety of cell signaling pathways. This study aims to examine the effects of expression of CHMP2B on other cell signaling pathways, particularly in the development of wings in Drosophila. We plan to express wild-type and mutant forms of CHMP2B in the wing using the GAL4-UAS system. Our preliminary results show that expression of wild-type CHMP2B appears to cause disjunction of the wing margin and deformation of the shape and veins on the wings.

A role for zebrafish Cxcl8-l2 in the regulation of neutrophil response to Influenza A virus infection

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Influenza A virus (IAV) infection has a major impact on human health and is responsible for over 200,000 hospitalizations and 36,000 deaths each year. The innate immune system recognizes and mounts the initial response to limit viral replication and spread. Recent studies suggest that neutrophils may contribute to host pathology and hyper-inflammation, but the underlying mechanisms remain unclear. Previous work in the Kim Laboratory introduced the zebrafish as a model for mammalian IAV infection. Cxcl8-l2, the zebrafish homologue of human CXCL8, is a potent neutrophil activator and chemoattractant that is upregulated during IAV infection. Cxcl8-l2 regulation of the innate immune response to IAV infection is studied using Cxcl8-l2 morphant zebrafish that could display altered survival, neutrophil localization, and proinflammatory cytokine levels compared to control zebrafish. This research can improve human health by by helping to identify future therapeutics and treatments to reduce the spread and the severity of IAV infections.

Role of Poised Enhancers in Lymphoid Cell Fate Determination

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Hematopoiesis involves gradual restriction of lineage differentiation potential from hematopoietic stem cells to mature blood cells. Lineage commitment and differentiation is regulated by specific interaction of pioneer transcription factors and co-regulators at enhancer elements to orchestrate gene expression. These enhancers acquire different chromatin modifications which serve as beacons for lineage specific transcription factors and chromatin modifiers. Recent evidence has shown correlation between priming of lineage specific enhancers in progenitor cells with activation of those enhancers during differentiation. We hypothesize that this enhancer priming step is functionally required for differentiation. To test this hypothesis, we will use genome and epigenome editing to alter primed enhancer elements in multipotent progenitors (MPPs) and evaluate their differentiation to the mature B lymphoid cell lineage. We will delineate how regulation of these enhancers leads to lymphoid specific gene expression profiles.

Insulin-like Growth Factor Binding Protein 4 is required for Adipogenesis and Skeletal Maturation in a gender-specific fashion

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Insulin-like Growth Factor (IGF) regulates bone growth and adipogenesis. Interestingly, IGF Binding Protein 4 (IGFBP4) is highly expressed in adipocytes and osteoblasts and was found to be inhibitory of IGF in vitro. However, studies suggested that it is not an inhibitor in vivo. Our purpose is to clarify how IGFBP4 mediates adipose and skeletal development in vivo in IGFBP4 null (IGFBP4$^{-/-}$) mice. Adult IGFBP4$^{-/-}$ mice suffered from growth retardation with reductions in weight, length, fat proportion and lean mass. White adipose tissues were smaller and Pparγ expression was significantly reduced in inguinal fat. Females, but not males, were protected against high fat diet-induced obesity, hypertrophy of adipocytes and liver steatosis. IGFBP4$^{-/-}$ females had marked reductions in bone density with decreased trabecular and cortical thicknesses. Surprisingly, males had significantly more trabecular bone. In summary, our results show that IGFBP4 modulates bone and fat development with a clear gender and tissue specificity.

JC Polyomavirus Enters Cells In a Dynamin-Dependent Clathrin-Mediated Endocytosis Manner

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JC polyomavirus (JCPyV) establishes a lifelong, persistent, asymptomatic infection in the kidney in the majority of the human population. In immunocompromised individuals JCPyV travels to the central nervous system causing progressive multifocal leukoencephalopathy (PML), a fatal, demyelinating disease. JCPyV requires α2,6-linked sialic acid receptors for attachment and 5–

hydroxytryptamine (5-HT)$_2$ receptors for internalization. JCPyV entry is thought to occur through clathrin-mediated endocytosis (CME), yet the cellular factors required for internalization have not been characterized. Clathrin-mediated endocytosis by 5-HT$_2$ receptors is mediated by interactions of specific scaffolding proteins with clathrin. Using specific small molecule inhibitors and dominant negative mutants we have demonstrated that JCPyV entry of target cells is mediated by CME and requires the molecular GTPase dynamin. These data demonstrate a deeper understanding of the cellular uptake mechanism utilized by JCPyV, providing new targets for possible therapeutics.

The role of Smad4 in the Foxd1 lineage during kidney development

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Receptor-regulated Smad proteins (R-Smads) are phosphorylated by transmembrane serine-threonine receptor kinases in response to TGF-β superfamily signaling. Activated R-Smad heteromeric complexes (Smad2/3 and Smad1/5/8) are translocated to the nucleus by the common mediator Smad (Smad4), where they regulate transcription of genes involved in a wide range of cellular functions including adhesion, migration, proliferation, differentiation, homeostasis, and apoptosis. Smad4 is believed to be non-redundant, and global knockout mice are embryonic lethal. Conditional gene inactivation studies to define the function of Smad4 in pericytes have been complicated by early embryonic lethality due to vascular defects. Organ-specific pericyte lineages have been identified, and Foxd1 is expressed in mural cell progenitors of the kidney. The Foxd1$^{+/Cre}$;Smad4 mouse model thus allows for conditional deletion of Smad4 in perivascular precursors without embryonic lethality. Lineage tracing with the ROSA26 reporter demonstrates that Smad4-deficient cells contribute to all expected perivascular compartments, including the glomerular mesangium. However, Foxd1$^{+/Cre}$; Smad4 mutant mice exhibit disorganized stroma with increased PDGFRβ and α-SMA levels in the medullary interstitium at postnatal day zero. 3D modeling of glomeruli is being used to understand if mesangial cells in mutant mice display a similar defect in organization that might affect convolution of the glomerular capillary.

Role of IL-17RD in vascular inflammation

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IL-17RD (SEF) is a transmembrane protein expressed in endothelial cells under physiological conditions and functions as an inhibitor of the fibroblast growth factor (FGF) signaling pathway.

Vascular inflammation is a process characterized by endothelial cell activation, which facilitates the infiltration of leukocytes across the vessel wall and leads to the formation of atherosclerotic plaques. Since IL17A is a proinflammatory cytokine involved in vascular inflammation, we postulated that IL-17RD modulates vascular inflammation either directly or through cross talk with other pathways. Preliminary results from our lab indicate that IL-17RD may function to modulate vascular inflammation in response to proinflammatory cytokines as well as proatherogenic stimuli such as cholesterol. Knockdown of IL17-RD regulates the expression of endothelial adhesion markers and affects monocyte adhesion to the endothelium. Ongoing experiments in our lab are focused on defining the interaction between IL-17RD and IL-17A to mediate this process.

Paclitaxel neurotoxicity correlates with keratinocyte-specific microtubule stabilization

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Paclitaxel is a microtubule-stabilizing chemotherapeutic agent. Despite its highly beneficial effects in cancer treatment, paclitaxel also induces sensory axon degeneration for which the mechanisms remain largely unknown. We previously demonstrated in zebrafish that paclitaxel promotes epithelial damage and upregulation of MMP-13 in basal keratinocytes within few hours of treatment. Axon degeneration is rescued upon MMP-13 inhibition, suggesting that skin-specific matrix changes underlie this phenotype. To analyze the mechanisms of MMP-13 upregulation, we assessed the role of paclitaxel-induced microtubule stabilization. Immunostaining for detyrosination (a measure of microtubule stability) during the course of 96h paclitaxel treatment, which typically induces axon degeneration, showed that microtubule structures were altered and detyrosination increased in keratinocytes, but not axons, starting around 48h of treatment. We conclude that microtubule stabilization likely does not contribute to increased MMP-13 expression, and although keratinocyte-specific alterations of microtubules are evident, these may not underlie axon degeneration.

Functional analyses of DICER co-factors in mammalian development and cancer

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MicroRNAs (miRNAs) are ~22 nucleotide non-coding RNAs that function as posttranscriptional regulators by modulating mRNA translation and stability. In the canonical miRNA biogenesis, DICER binds two double-stranded RNA binding protein co-factors, TARBP2 and PRKRA, which modulate pre-miRNA “dicing” kinetics, cleavage site selection and loading of the miRNA duplex into the RNA induced silencing complex (RISC). Furthermore, recent studies with the small molecule enoxacin reveal a cancer cell-specific growth inhibition response mediated by the

DICER co-factor TARBP2. We have tested the in vivo function of the DICER co-factors in the context of mammalian development as well as the target specificity of enoxacin using mouse cell lines genetically ablated for the DICER co-factors. Our study suggest that these cofactors are not functionally redundant and that TARBP2 and PRKRA may be important for processing specific classes of miRNA's at certain stages or have a novel function in post-transcriptional regulation apart from miRNA biogenesis.

Cell Cycle Progression of P-Granule Protein PGL-1 Segregation During Early Embryo Development in Caenorhabditis elegans

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Utilizing the nematode Caenorhabditis elegans as our model organism, we looked at the cell cycle progression and P-granule segregation in early embryos. P-granules are a class of perinuclear protein/ribonucleic acid (RNA) granules which follow the formation of the germ line in our model organism. Proteins such as PGL-1, which are responsible for binding RNA, are components of the P-granule. We stained C. elegans embryos using immunofluorescence with antibodies for tubulin and PGL-1. We discovered that, during mitosis PGL-1 condensed to one side of the cell, beginning at prophase. By metaphase the PGL-1 had migrated to the distal side of the spindle pole in one of the daughter cells. We propose that this pattern of segregation within the cytoplasm as chromosomes are moving towards the metaphase plate contributes to the successful asymmetric segregation of the P-granules to only one daughter cell.

Assessing healthspan in C. elegans: lessons from short-lived mutants

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Extended lifespan is often positively associated with stress resistance stress and somatic maintenance. Recently, a provocative study presented a binary model for healthspan and

concluded that well-characterized long-lived C. elegans mutants spend an increased proportion of life in a frail state when compared physiologically. However, measuring healthspan in invertebrate models suffers from confounders arising from standardization in experimental approach and definition of becoming "geriatric". In the current study, we compared wild-type worms and short-lived mutants displaying either accelerated aging or chronic illness to categorize parameters of health as age-dependent or merely age-related. To assess the proportionality between lifespan and healthspan, we proposed a straightforward method of empirically determining the geriatric state using the steepest rate of decline in health as onset of gerospan. Using this definition, we demonstrate that accelerated aging results in a reduced gerospan when compared to wild-type physiologically. We propose that this non-proportionality is due to extrinsic aging factors which scale with time and discuss the limitations of physiological comparisons.

Triclosan is a Mitochondrial Uncoupler in Live Zebrafish

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Triclosan (TCS) is a synthetic antimicrobial agent used in many consumer goods, thus has been detected widely in humans. Here we present novel data that TCS is also a mitochondrial uncoupler in a living organism: 24 hpf zebrafish embryos. These experiments were conducted using a Seahorse Bioscience XF 96 Analyzer modified for bidirectional temperature control, using the spheroid plate to measure one zebrafish embryo per well. Using this method, acute exposure to TCS increases basal oxygen consumption rate without affecting mortality. TCS also decreases ATP-linked respiration and spare respiratory capacity and increases proton leak: all indicators of TCS being a mitochondrial uncoupler in vivo. This is the first example of usage of a Seahorse Analyzer to measure bioenergetic flux of a single zebrafish embryo per well in a 96 well format. The method developed in this study provides a high-throughput tool to identify previously-unknown mitochondrial uncouplers in a living organism.

MicroRNA Let-7i enhances heart tissue regeneration in zebrafish

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Humans have limited capacity to regenerate heart muscle following injury. In contrast, zebrafish are capable of regenerating myocardium, resolving scar tissue and restoring heart function. In this study, we investigate the role of the highly upregulated microRNA let-7i, during heart

regeneration. Depletion of let-7i function using antisense oligonucleotides at the onset of injury resulted in defective epicardium migration, as revealed by the Tg(tcf21:RFP) reporter strain, and attenuated cardiomyocyte proliferation indices. Conversely, increased let-7i activity with a heat-inducible transgenic strain, Tg(hsp70:let-7ipre), enhanced cardiomyocyte proliferation indices by ~30%. Fluorescence-activated cell sorting of wildtype tissue revealed that microRNA let7i is enriched by ~3fold within epicardial cells compared to other cell types of the heart such as cardiomyocytes and endothelial cells. In contrast to mouse heart injury studies, our zebrafish data indicate that injury-induced upregulation of let-7i is critical for epicardium migration and proliferation of spared cardiomyocytes, two critical processes for heart regeneration.

Defining the role of cellular Ca^{2+} in JCPyV attachment and infection

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JC polyomavirus (JCPyV) establishes infection through fecal-oral transmission and remains dormant in the kidney of healthy individuals. In immunocompromised individuals, JCPyV migrates to the central nervous system (CNS), resulting in the fatal demyelinating disease progressive multifocal leukoencephalopathy (PML). Currently there are no effective treatments or therapies for JCPyV or PML. Virus-host cell interactions regulate the infectious process and critically influence viral pathogenesis. JCPyV attachment is mediated by binding to α2,6-linked LSTc, while internalization is mediated by serotonin receptors (5-HT_2Rs). 5-HT_2Rs induce intracellular calcium (Ca^{2+}) release upon ligand binding to activate signaling pathways. Inhibition of Ca^{2+} release from the ER reduces JCPyV infection; demonstrating that intracellular Ca^{2+} flux is necessary for infection. However, virus binding was not affected by modulation of Ca^{2+} flux as measured by flow cytometry. Current studies focused on defining how modulation of intracellular Ca^{2+} regulates JCPyV infection will provide important insights into JCPyV pathogenesis.

An in vivo, cell type- and compartment-specific analysis of translation in motor neurons of two mouse models of Charcot-Marie-Tooth disease Type 2D

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How dominant mutations in glycyl tRNA synthetase (GARS) cause Charcot-Marie-Tooth disease Type 2D (CMT2D) peripheral neuropathy is completely unknown. The technical challenge of studying the mammalian peripheral axon in vivo has contributed to the lack of a disease mechanism. The goal of this project is to use an in vivo, cell type- and compartment-specific approach to test the hypothesis that mutations in Gars cause impaired translation in

motor neurons of two mouse models of CMT2D. Non-canonical amino acid tagging, ribosome tagging, and thiouracil-tagging will be used to create a comprehensive profile of translation and transcription in CMT2D, healthy wild-type, and regenerating wild-type motor neuron cell bodies and axons**.** This study will test a valid disease mechanism as well as contribute new knowledge about the basic biology of motor neurons, with specific emphasis on the axonal compartment.

Human CHMP2B^{Intron5} can cause cell fate transformations when expressed during the development of Drosophila external sensory organs.

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The human CHMP2B gene encodes a member of the ESCRT-3 complex and plays a major role in endosomal vesicle traffic, including the recycling and degradation of cell surface receptors. A variant form of CHMP2B (CHMP2B^{Intron5}) is associated with the development of frontotemporal dementia. Using Drosophila melanogaster as a model, we have previously shown that misexpression of the human CHMP2B^{Intron5} variant in Drosophila eyes can disrupt the normal function of cell signaling pathways and lead to neuordegeneration of the photoreceptors. To further investigate the activity of CHMP2B^{Intron5} on cell signaling processes, we have misexpressed this variant form during the development of the external sensory organs. We observe that CHMP2B^{Intron5} can cause cell fate transformations – specifically, a strong shaft cell to socket cell transformation as a strong effect and a neuron to sheath cell transformation as a weaker effect. Both of these effects are reminiscent of increases of Notch activity by gain-of-function mutations, for example. We are currently testing the hypothesis that CHMP2B^{Intron5} is leading to an increase in Notch pathway activity by assessing the ability of Notch pathway mutations to either suppress or enhance the CHMP2B^{Intron5} phenotype.

Role of Tbx1 in Late Stage Stria Vascularis Development

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Early studies have shown that Tbx1 play important roles for inner ear development during embryonic stage. In this study, we will apply a hypomorphic Tbx1^{wdml} mutation to investigate a previously unknown role of TBX1 in regulating late-stage inner ear development. We found that Tbx1^{wdml} mice are deaf due to abnormal stria vascularis development. We hypothesize that TBX1 plays an important role during late stage inner ear development by regulating the maturation of non-sensory epithelial cells in the stria vascularis, and the Tbx1^{wdml} missense mutation prevents this late stage function. We found Tbx1 mRNA and protein expression in stria vascularis. Immunostaining and in-situ hybridization with markers specific for stria vascularis

cell layers indicate absence of marginal cells. We will apply yeast two hybrid assay to identify proteins interact with TBX1 during this stage. This study will lead to discovery of novel roles of TBX1 during late stage stria vascularis development and identify other proteins that are involved in this process.

Effects of chondroitin proteoglycans on the development of C. elegans

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One suggested culprit of cancer development is a series of mutations in glycosylated proteins, proteoglycans, in the extracellular matrix. Glycosylation plays a key role in development and physiological processes. Mutations causing abnormal glycosylation can over-activate certain cell signaling pathways, which promotes tumor development (Yamamoto et al., J. of Cancer, 106, 2012). One of the most common glycan chains is chondroitin sulfate, which may be necessary for cytokinesis during embryonic cell division (Shim & Paik, Proteomics, 10:846-857, 2010). We therefore studied chondroitin proteoglycan-2 (CPG-2) in C. elegans. The transcript encoding CPG-2 is one of the most abundant in the germline and translation occurs during oocyte maturation. In order to examine the effects of CPG-2 on development, we created CRISPR/Cas9 knock-ins with fluorescently-tagged CPG-2. After cloning the sgRNA and GFP::CPG-2 vectors, we injected wild type C. elegans with sgRNA and cpg-2 plasmid DNA and observed the effects on embryonic and organismal development.